

Shade-induced stem elongation in rice seedlings: Implication of tissue-specific phytohormone regulation

Summary A better understanding of shade avoidance syndrome (SAS) is an urgent need because of its effect on energy reallocation. Leverage-related mechanism in crops is of potential economic interest for agricultural applications. Here we report the SAS phenotype at tissue level rice seedlings. Tissue-specific RNA-sequencing indicates auxin plays different roles between coleoptile and the first leaf. Phenotypes of wild type treated by gibberellin and brassinosteroid biosynthesis inhibitors and of related mutants suggest these two hormones positively regulate SAS. Our work reveals the diversity of hormone responses in different organs and different species in shade conditions.

Plants growing at high density experience a low red to far-red light ratio (R/FR) and a reduction in the amount of photosynthesis active radiation (PAR, between 400 nm and 700 nm), which triggers a series of physiological changes termed a shade-avoidance response or syndrome (SAS). A common phenotype of SAS is out-growing neighborhoods while deprioritizing of harvestable organs, which would compromise the yield of crops (Franklin and Whitelam 2005). The detailed phenotypes and mechanisms of SAS have been mainly characterized in dicots (*Arabidopsis*, *Brassica rapa*, tobacco, tomato etc.) (Tao et al. 2008; Casal 2013; Procko et al. 2014); however, little information in this regard is available in monocots (Kebrom and Brutnell 2007). Rice, as the primary staple for more than half the world's population, is of economic interest to plants in high density. Here we examined SAS in coleoptile, the first leaf, the second leaf sheath and the shoot of rice seedlings. The results from experiments: (i) Gene Ontology (GO) enrichment analysis of shade regulated genes based on tissue-specific RNA-sequencing data; (ii) phenotype of seedlings treated by the inhibitor of auxin transporter, the inhibitors of GA and BR biosynthesis; and (iii) phenotype of GA- and BR-related mutants under shade, indicate GA and BR are positively involved in SAS of all aerial parts, but auxin plays different roles in coleoptile and the first leaf.

Rice has three phytochrome genes (*PHYA*, *PHYB* and *PHYC*) in the genome (Takano et al. 2005). It has been reported that the lamina joint inclination of rice seedlings increased in reduced R/FR condition and *OsphyB* acts as a sole photoreceptor in this response (Shin and Park 2014). Here, we monitored the length of coleoptile, the first leaf, the second (2^{nd}) leaf sheath and the shoot of rice seedlings grown under shade conditions (low R/FR and low PAR). Rice wild-type seeds (*Oryza sativa* L. cv. Nipponbare) were grown on semi-solid (0.4% agar in water) under continuous white

light for 1 d, and then maintained in white light or transferred to shade for 4 d. Compared with white light-grown seedlings, the lengths of coleoptile were increased by 97% in shade. The increased elongation were also observed on the first leaf (by 69%), on the second leaf sheath (by 58%) and the shoot (by 42%) after 4 d shade (Figure 1A). The similar phenotypes of T65 and Zh11 seedlings under shade are shown in Figure S1.

To dissect the effect of low PAR and low R/FR on growth, we detected the phenotype of wild type seedlings under white light (high R/FR) with the reduced PAR levels (Figure S2). The change in light quality (R/FR) appears predominant for SAS phenotype under our conditions. We also found the length of coleoptile increased in response to decreased R/FR (0.9, 0.67 and 0.33) at the same PAR (Figure S3).

Transcriptional studies have been used to discover the mechanism of SAS. In order to figure out the tissue-specific mechanism in rice, we collected coleoptiles and the first leaves separately from the seedlings treated by 1 h shade or white light for RNA sequencing. There are 251 shade upregulated and 1,257 downregulated genes (Fold Change > 2, $P < 0.01$) in coleoptiles (Table S1) and 276 shade induced and 464 repressed genes in the first leaves (Table S2; Figure 1B). Only 14 shade induced genes and 95 shade repressed genes are overlapped in two tissues. Gene ontology (GO) analysis shows “unidimensional cell growth” and “gibberellin biosynthetic process” are enriched in both shade upregulated genes of coleoptiles and the first leaves. Interestingly, “response to auxin stimulus” related genes appear repressed in coleoptiles but induced in the first leaves (Figure 1B; Tables S3, 4), indicating a tissue-specific regulation of auxin.

Phytohormones such as auxin, gibberellins and brassinosteroids have been reported to facilitate shade induced hypocotyl elongation in *Arabidopsis* (Casal 2013). But the regulations of these hormones by shade in rice remain unexplored. To verified RNA-sequencing data (Table S5), we detected the expression levels of auxin responsive genes (*OsIAA20* and *OsGH3;1*), GA biosynthetic genes (*OsGA20ox1* and *OsGA20ox2*) and BR responsive genes (*OsDWARF* and *OsBRI1*) after 1 h shade treatment in coleoptiles and the first leaves (Figure 1C). Consistent with that in *Arabidopsis*, the inductions of GA biosynthesis genes (*OsGA20ox1* and *OsGA20ox2*) and the transcriptional regulations of BR responsive genes (*OsDWARF* and *OsBRI1*) indicate an enhanced GA and BR response under shade in both tissues of rice seedlings. While BR related GO terms didn't show up in the web based GO enrichment analysis, when we picked up a cluster of rice putative BR responsive genes based on homology from our RNA-sequencing dataset (Vriet et al. 2015) (Table S6), significant induction of these genes in shade suggested the increased BR response in coleoptiles

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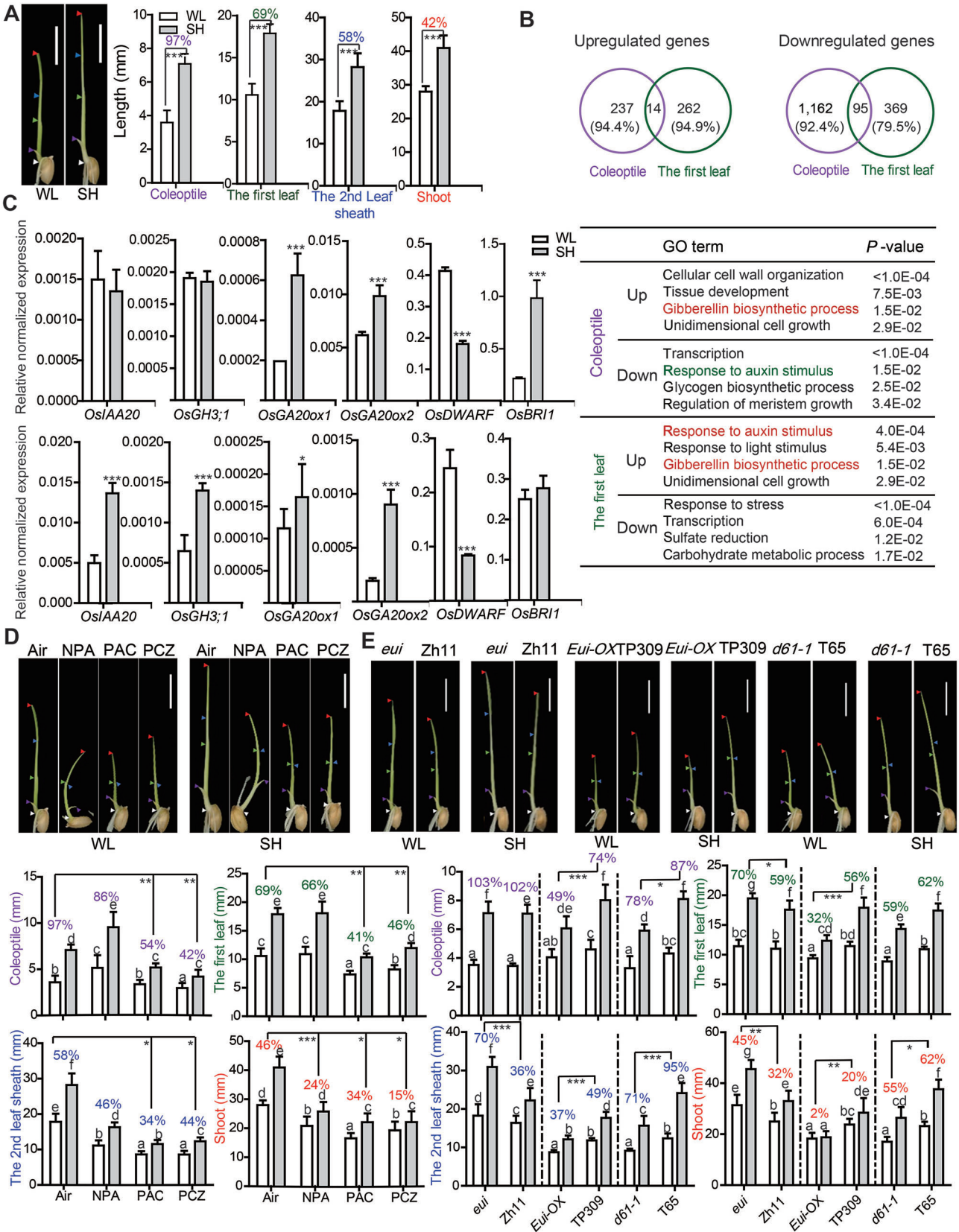


Figure 1. Continued.

and the first leaves (Figure S4, $P < 0.05$). Moreover, the expression of cell wall organization gene *OsXTH24* encoding xyloglucan endotransglycosylase is also induced by shade (Figure S5), consistent with the elongation growth in coleoptiles and the first leaves.

Despite being induced in the first leaves of rice, as that in *Arabidopsis*, the expression of *OsIAAs* and *OsGH3;1* is unchanged in coleoptiles after shade treatment, confirming the tissue-specific regulation of auxin.

To further confirm the effects of auxin, GA and BR on shade induced stem elongation, we cultured rice seedlings with NPA (1-N-Naphthylphthalamic acid, an auxin polar transport inhibitor), PAC (Paclobutrazol, a GA biosynthesis inhibitor) and PCZ (Propiconazol, a BR biosynthesis inhibitor) (Figure 1D). After NPA treatment, the aerial parts of seedlings grow in a curly manner and the roots lose gravitropism as previously reported. While NPA treatment increases coleoptile length, it has little effect on the first leaf elongation but decreases the length of the second leaf sheath and the shoot under both white light and shade. Compared to the mixed response by NPA treatment, PAC- and PCZ-treatment uniformly suppressed shade induced elongation of coleoptile, the first leaf, the second leaf sheath and the shoot, suggesting the positive role of GA and BR in SAS. This is consistent with that brassinosteroid biosynthesis inhibitor brassinazole decreases the shade response on lamina joint inclination (Shin and Park 2014). The supplement of GA_3 can rescue the effect of PAC on coleoptile under shade (Figure S6), confirmed GA is required for shade-induced elongation response in rice.

We also verified the function of GA and BR in shade using genetic mutants. It has been reported that *Eui* gene, a P450 monooxygenase that inactivates GAs, is involved in GA homeostasis at the seedling stage (Zhu et al. 2006). *eui* mutants exhibit elongated phenotypes due to accumulation of GA_1 and GA_4 and the *Eui*-OX plants are dwarf. When we grew them in shade, *eui* mutants displayed the enhanced shade-induced elongation compared with wild type Zh11 on the first leaf, the second leaf sheath and the shoot; *Eui*-OX seedlings exhibited the reduced elongation growth on all aerial parts after shade treatment compared with wild type TP309 (Figure 1E). The positive effect of BR is confirmed by rice BR-hyposensitive mutant *d61-1* (a BR receptor mutant) (Yamamoto et al. 2000). When growing in shade, *d61-1* exhibits shorter coleoptile, the first leaf, the second leaf sheath and the shoot compared to wild type control T65 (Figure 1E). Statistical analysis of the percentage increase by shade

compared to wild type reveals defective shade response in most aerial parts except in the first leaf.

Auxin, as we have seen in previous studies, plays a major role in SAS in the model dicot, *Arabidopsis* (Tao et al. 2008). However, the expression levels of selected auxin responsive genes are not increased in rice coleoptile by shade treatment (Figure 1B, C). Together with the effect of NPA on coleoptile (Figure 1D), it is possible that auxin functions differentially between coleoptile in rice and hypocotyl in *Arabidopsis* under shade. The growth inhibitions by NPA are shown in the second leaf sheath and the shoot. The effects of NPA are largely dependent on where auxin is generated and transported to. This compartment-specific effect warrants more research for insights.

The crosstalk between light and hormones has been studied in rice seedlings. OsphyB acts as a negative regulator of BL-regulated growth (Jeong et al. 2007) and mediates the repression of GA biosynthesis capacity (Hirose et al. 2012). Whether the shade phenotype is due to endogenous hormone biosynthesis or hormone signaling sensitivity, exogenous application of hormone on the hormone biosynthesis mutants may help.

In the present study, we characterized the phenotypes of SAS in rice seedlings and investigated the possible functions of phytohormones (auxin, GA and BR) under shade. We show GA and BR signals contribute to shade induced stem elongation and the role of auxin may vary in different tissues.

It is known that GA biosynthesis inhibitors can be used to form compact seedlings in farming technology. Our results propose potential novel applications of the inhibitors to dampen SAS.

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Figure 1. Shade-induced stem elongation in rice seedlings

(A) Phenotypes of wild type rice seedlings grown under white light and shade condition. White arrowhead points to the node of coleoptile. The arrowheads with the different color indicate the end of the coleoptile (purple), the first leaf (green), the second leaf sheath (blue) and the shoot (red). White scale bar = 10 mm. The percentage of increase by shade is listed above the column. (B) The numbers and GO analysis of differential expressed genes in coleoptiles and the first leaves. (C) Expression level of hormone related genes regulated by shade in coleoptiles (top panel) and the first leaves (bottom panel) by qRT-PCR. (D) Effects of NPA, PAC and PCZ on shade induced growth. Bars marked with different letters denote significant differences ($P < 0.05$) of the means of the length. The significant differences on the percentage increase by shade compared in air condition is shown as asterisks. White scale bar = 10 mm. (E) Phenotypes of *eui* (Zh11 is wild type cultivar), *Eui*-OX (TP309 is wild type cultivar) and *d61-1* (T65 is wild type cultivar) under white light and shade. Bars marked with different letters denote significant differences ($P < 0.05$) of the means of the length. The significant differences on the percentage increase by shade compared to wild type is shown as asterisks. * $P < 0.01$, ** $P < 0.05$ and *** $P < 0.001$, calculated by student's t-test. White scale bar = 10 mm.

Keywords: Auxin; BR; GA; rice seedlings; shade avoidance response

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AUTHOR CONTRIBUTIONS

H.L. detected the phenotype of rice seedlings. C.Y. performed RNA-sequencing analyses. L.L. designed the experiment, supervised the study, drafted and revised the manuscript.

REFERENCES

- Casal JJ (2013) Photoreceptor signaling networks in plant responses to shade. *Annu Rev Plant Biol* 64: 403–427
- Franklin KA, Whitelam GC (2005) Phytochromes and shade-avoidance responses in plants. *Ann Bot* 96: 169–175
- Hirose F, Inagaki N, Hanada A, Yamaguchi S, Kamiya Y, Miyao A, Hirochika H, Takano M (2012) Cryptochrome and phytochrome cooperatively but independently reduce active gibberellin content in rice seedlings under light irradiation. *Plant Cell Physiol* 53: 1570–1582
- Jeong DH, Lee S, Kim SL, Hwang I, An G (2007) Regulation of brassinosteroid responses by phytochrome B in rice. *Plant Cell Environ* 30: 590–599
- Kebrom TH, Brutnell TP (2007) The molecular analysis of the shade avoidance syndrome in the grasses has begun. *J Exp Bot* 58: 3079–3089
- Procko C, Crenshaw CM, Ljung K, Noel JP, Chory J (2014) Cotyledon-generated auxin is required for shade-induced hypocotyl growth in *Brassica rapa*. *Plant Physiol* 165: 1285–1301
- Shin J, Park PB (2014) Shade avoidance and the regulation of leaf inclination in rice. *Rapid Commun Photosci* 3: 53–55
- Takano M, Inagaki N, Xie X, Yuzurihara N, Hihara F, Ishizuka T, Yano M, Nishimura M, Miyao A, Hirochika H, Shinomura T (2005) Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. *Plant Cell* 17: 3311–3325
- Tao Y, Ferrer JL, Ljung K, Pojer F, Hong F, Long JA, Li L, Moreno JE, Bowman ME, Ivans LJ, Cheng Y, Lim J, Zhao Y, Ballare CL, Sandberg G, Noel JP, Chory J (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* 133: 164–176
- Vriet C, Lemmens K, Vandepoele K, Reuzeau C, Russinova E (2015) Evolutionary trails of plant steroid genes. *Trends Plant Sci* 20: 301–308
- Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S, Ashikari M, Kitano H, Matsuoka M (2000) Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. *Plant Cell* 12: 1591–1606
- Zhu Y, Nomura T, Xu Y, Zhang Y, Peng Y, Mao B, Hanada A, Zhou H, Wang R, Li P, Zhu X, Mander LN, Kamiya Y, Yamaguchi S, He Z (2006) ELONGATED UPPERMOST INTERNODE encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. *Plant Cell* 18: 442–456

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Supplemental experimental procedures

Figure S1. Phenotypes of T65 and Zh11 grown under white light and shade condition

White arrowhead points to the node of coleoptile. The arrowheads with different colors indicate the end of the coleoptile (purple), the first leaf (green), the second leaf sheath (blue) and the shoot (red). White scale bar=10mm. The percentage of increase by shade is listed above the bars.

Figure S2. Phenotypes of wild type rice seedlings grown under white light with different PAR levels and shade conditions. White arrowhead points to the node of coleoptile. The arrowheads with the different color indicate the end of the coleoptile (purple), the first leaf (green), the second leaf sheath (blue) and the shoot (red). White scale bar = 10 mm. The PAR levels are listed above the pictures. Shade condition: Red, $16 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; Far Red, $48 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; Blue, $1.3\text{--}1.5 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Figure S3. Phenotypes of wild type rice seedlings grown under white light and shade with different R/FR conditions

The arrowheads indicate the end of the coleoptile. White scale bar=10mm. The ratio of R/FR is listed above the pictures. The percentage increase by shade is listed above the dots in line chart.

Figure S4. Boxplot represents the expression level of BR responsive genes in coleoptiles and the first leaves with or without shade treatment (the expression level are shown in Table S6). Significant difference was calculated by t-test between white light and shade samples.

Figure S5. Expression level of OsXTH24 in coleoptiles and the first leaves by qRT-PCR

Figure S6. Phenotypes of wild type rice seedlings grown with different concentrations of PAC and GA₃

The arrowheads indicate the end of the coleoptile. White scale bar = 10mm. The concentrations of PAC and GA₃ are listed above the pictures. The percentage increase by shade is listed above column in histogram.

Table S1. The expression levels of differentially expressed genes in coleoptile after 1 h shade treatment (FPKMs (Fragments per kilobase of exon per million fragments mapped) were listed.

Table S2. The expression levels of differentially expressed genes in the first leaf after 1 h shade treatment (FPKMs (Fragments per kilobase of exon per million fragments mapped) were listed.

Table S3. Over-represented GO terms of differentially expressed genes in coleoptile after 1 h shade treatment (Terms with $P < 0.05$ are shown)

Table S4. Over-represented GO terms of differentially expressed genes in the first leaf after 1 h shade treatment (Terms with $P < 0.05$ are shown)

Table S5. The expression levels of examined genes in Figure 1C based on RNA-sequencing data (FPKMs (Fragments per kilobase of exon per million fragments mapped) were listed.

Table S6. The expression levels of BR-related genes in coleoptile and the first leaf after 1 h shade treatment (FPKMs (Fragments per kilobase of exon per million fragments mapped) were listed.

Table S7. The primers used in this study